SELECTIVE BINDING OF ORGANOPHOSPHATE PESTICIDES USING MOLECULAR IMPRINTED POLYMERS.

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ABSTRACT

Molecular Imprinted Polymers (MIPs) have been used for recognition and binding of different compounds. We are developing MIPs to selectively bind organophosphate pesticides and toxic chemical warfare nerve agents. MIPs were made to the pesticide Dichlorvos. Control MIPs were made without the template. Dichlorvos-MIPs bound more Dichlorvos in comparison to control-MIPs. The Dichlorvos-MIPs can be regenerated after binding the pesticide and reused at least three times by repeating the washing procedure. Dichlorvos-MIPs demonstrated specificity for its template since these MIPs did not bind Methamidophos, Phosdrin, and Metasystox I, compounds with similar chemical structures to that of Dichlorvos.

INTRODUCTION

Molecular Imprinted Polymers (MIPs) are highly cross-linked polymers, which are formed by cross-linking monomer in the presence of a template. The template molecule interacts with the monomer (usually methacrylic acid for a basic template molecule and divinyl pyridine for an acidic template molecule) in solution either covalently or non-covalently and highly cross-linked polymers form around the template. For example, a template molecule is dissolved in a progen (solvent), the template self-assembles with the monomer when mixed with cross-linker and polymerizes with the addition of an initiator. Following removal of the template molecule, the resultant polymers possess the steric and chemical memory for the recognition of that template (Figure 1).

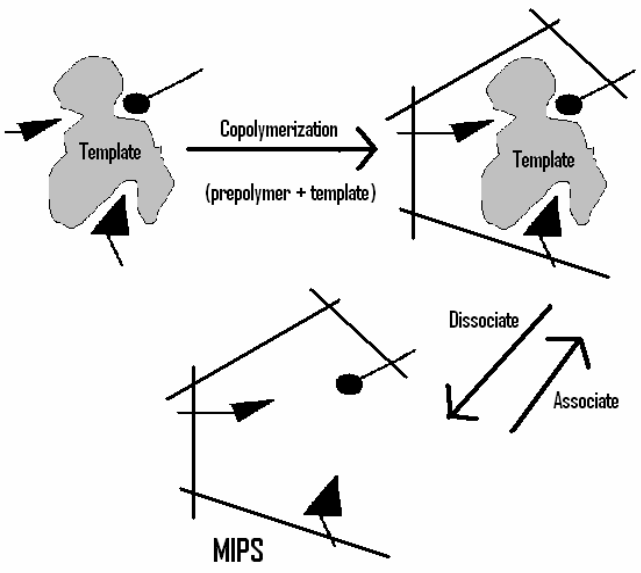

Figure 1. Formation of MIPs.

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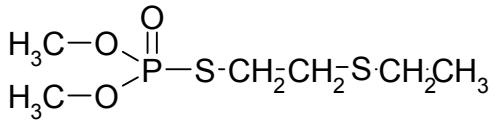
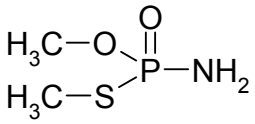
MIPs can theoretically be produced to mimic receptors, enzymes, and antibodies, and can be used for decontamination of toxic compounds[1-9]. Antibodies, enzymes, and receptors posses natural specific recognition elements, which can be used in enzyme assays, immunoassays, as a biosensor, and various affinity methods. These biological reagents, however, suffer from low stability and high product costs. However, some immobilization processes improve biosensor stability (Gordon[10-11] et al). Analytical methods such as high-performance liquid chromatography, gas chromatography, nuclear magnetic resonance, and mass spectrophotometry are useful and specific techniques for quantifying many compounds such as sugars, amino acids, drugs, and pesticides[12-15]. Yet these techniques need expensive and bulky equipment and specialized personnel. In addition, field units are not practical. MIPs have advantages over these methods, such as tolerance to high thermal conditions, extreme pH, insensitivity to organic solvents, and extremely long shelf-like without any need for special storage conditions[16-22].

MATERIAL AND METHODS

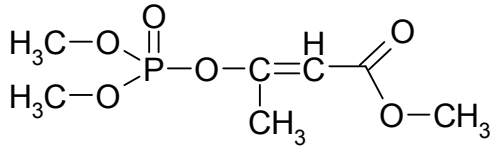
Materials. 4-Vinylpyridine, ethylene glycol dimethacrylate, and 2,2'-azobisisobutyronitrile were from Aldrich Chemical Company (Milwaukee, WI). Dichlorvos, Methamidophos, Phosdrin, and Metasystox I were from ChemService Inc (West Chester, PA); the chemical structures of these pesticides are shown in Figure 2. Acetylcholinesterase (AChE) was purified from fetal bovine serum as previously described[23]. All other materials were obtained from commercial supplies and used without further purification. The reagent grade chemicals were dissolved in deionized water to prepare solutions.



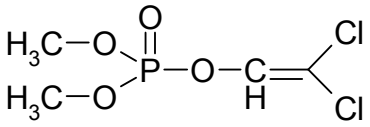
Metasystox I



Methamidophos



Phosdrin



Dichlorvos

Figure 2. Chemical structure of the pesticides used in the MIPs binding assays

Preparation of MIPs. MIPs were made according to the method described by Baggiani et al[24]. In a 20 mL glass scintillation vial, 3.2 mL of methanol-water (3:1 v/v) was added. Two hundred mg Dichlorvos (template) was added to methanol-water and mixed. This was followed by adding 0.243 mL (2.35 mM) 4-vinylpyridine, 2.95 mL (15.7 mM) ethylene glycol dimethacrylate, and 40 mg (0.24 mM) 2,2'-azobisisobutyronitrile. The mixture was purged with nitrogen gas and sonicated in a water-bath for 5 minutes. The mixture was purged with nitrogen gas again and polymerized for 14 hours at 60^{o}C. Control-MIPs were made in the same manner but without Dichlorvos (template). The polymers were kept at 4^{o}C for at least one hour. The polymers were then broken with a hammer and mechanically ground by a mortar grinder. The polymers were washed extensively with 0.1 N NaOH-methanol (1:1, v/v) to hydrolyze Dichlorvos, followed by acetic acid-methanol (1:9, v/v) to release the unhydrolyzed and hydrolyzed template, and ethanol-water (1:1, v/v) to expose the template binding sites. MIPs were then dried at 80^{o}C until the solvent was evaporated and used for binding assays.

Analysis of Binding of Dichlorvos to MIPs by a Back-Titration Assay Using AChE. The extent of binding of Dichlorvos to MIPs was determined by a back-titration assay using purified AChE. Enzyme assays were performed as previously described[25] using acetylthiocholine (0.83 mM) as substrate. Dichlorvos was mixed with MIPs for two hours, centrifuged, and the unbound Dichlorvos in the supernatant was added to each well of a microtiter plate. Ethanol, which was used in the binding assays, was evaporated at 37^{o}C. This was followed by adding AChE to a final volume of 50 uL to each well of a microtiter plate. The mixture was incubated for one hour at room temperature. Two hundred fifty uL of substrate (acetylthiocholine) in 50 mM phosphate buffer, pH 8.0 was then added and the enzyme activity was measured at 412 nm for 10 minutes at room temperature in a microtiter plate reader. Each assay was performed in five identical wells of a microtiter plate and each experiment was repeated at least twice.

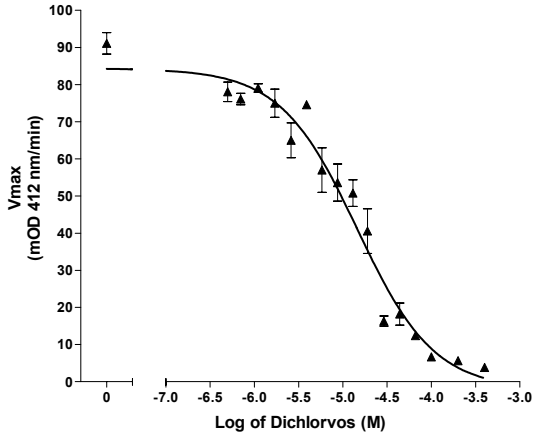


Figure 3A

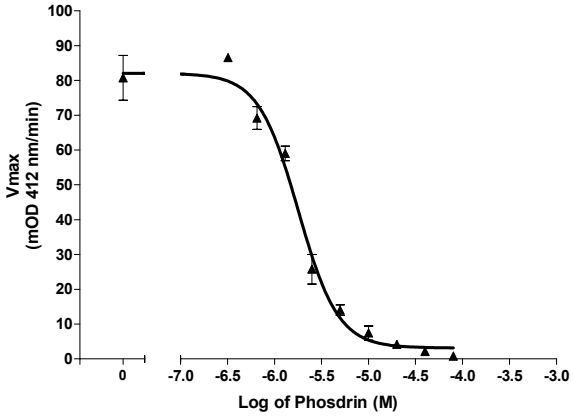


Figure 3B

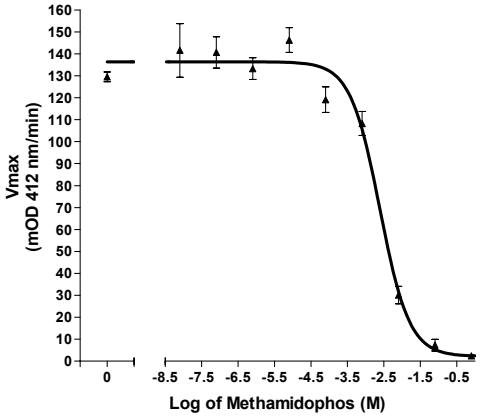


Figure 3C

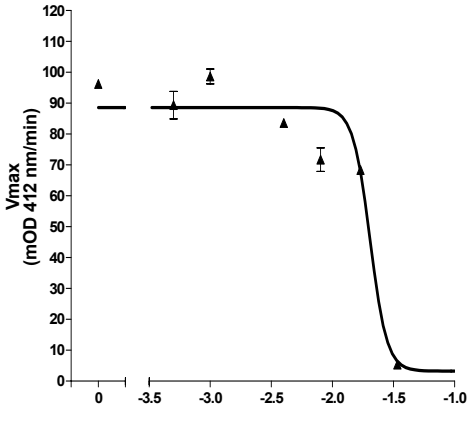


Figure 3D

Figure 3. Effect of different concentrations of Dichlorvos (3A), Phosdrin (3B), Methamidophos (3C), and Metasystox I (3D) on AChE activity.

RESULTS and DISCUSSION

Inhibition of AChE by Dichlorvos and other pesticides with similar chemical structures as Dichlorvos. In order to determine the optimal concentration of the pesticides for the binding assays, different concentrations of these pesticides were used in the kinetic assays using AChE. Concentrations of each pesticide are dissolved in ethanol, and 40 uL of the solution was assayed as discussed in Materials and Methods. Enzyme without the pesticides was also used to measure the total activity of the enzyme (control). The concentrations of these pesticides, which inhibited enzymatic activity of AChE, were used in the MIPs binding assays.

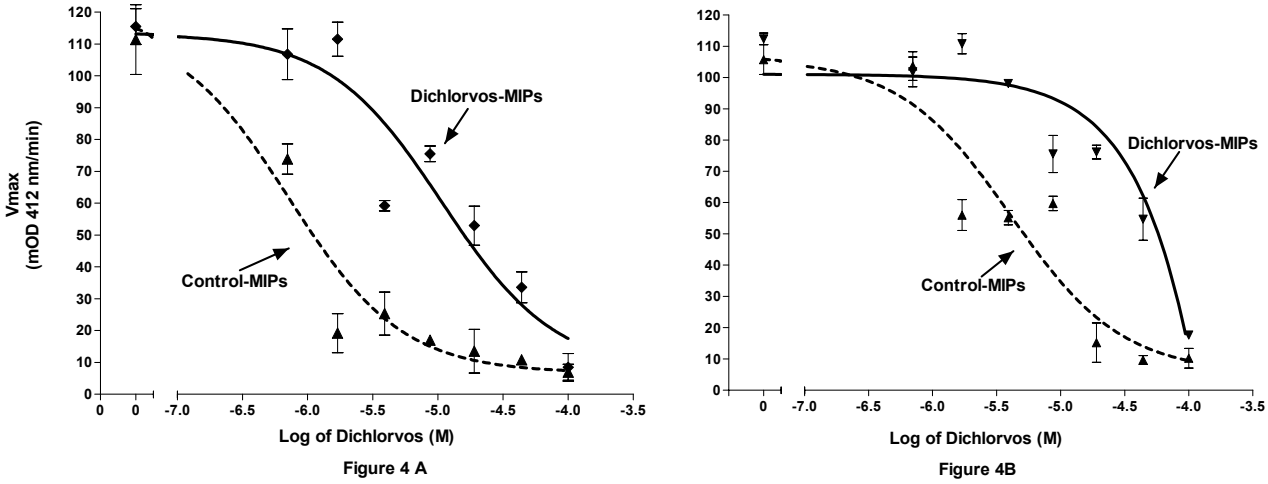


Figure 4A. Binding of Dichlorvos to Dichlorvos- and control-MIPs. Figure 4B. Binding of Dichlorvos to regenerated Dichlorvos- and control-MIPs. AChE activity was used to measure the binding of Dichlorvos to MIPs.

We synthesized MIPs that bound to their corresponding template (Dichlorvos). In order to test the specificity of Dichlorvos-MIPs, we used pesticides with similar chemical structures to that of Dichlorvos (Figure 2) for binding to these MIPs. The chemical structures of these pesticides were similar to the phosphate group of the structure of Dichlorvos (Phosdrin and Metasystox I). We also compared Methamidophos, a pesticide smaller than Dichlorvos. The inhibitory effects of these three pesticides and Dichlorvos on AChE are shown in Figure 3. Figure 3A shows that Dichlorvos dose-dependently inhibited AChE. Similarly, Figure 3B shows that Phosdrin dose-dependently inhibited AChE, but Phosdrin was a more potent inhibitor of AChE than Dichlorvos. Figures 3C and 3D show that Methamidophos and Metasystox I were both less potent inhibitors of AChE than Dichlorvos. Thus, the order of inhibition of these pesticides was Phosdrin > Dichlorvos > Methmidophos > Metasystox I.

Figure 4A shows that Dichlorvos bound more to the Dichlorvos-MIPs in comparison to control-MIPs; the latter synthesized in the absence of template. Notably, Dichlorvos bound to Dichlorvos-MIPs only when ethanol was the solvent. No specific binding was observed when the solvents were: water, phosphate buffer, tris buffer, or methanol (data not shown). Figure 4B shows that the MIPs, which had been reused three times, bound the template (Dichlorvos) as well as fresh MIPs (Figure 4A). Therefore, the MIPs are reusable.

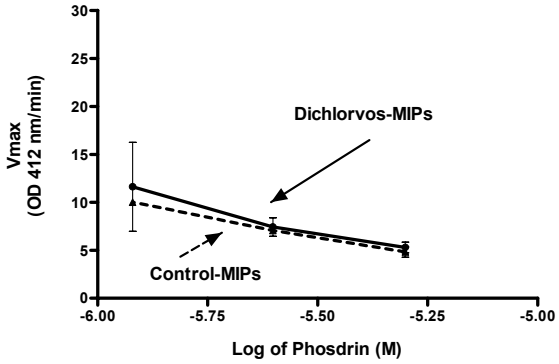


Figure 5A

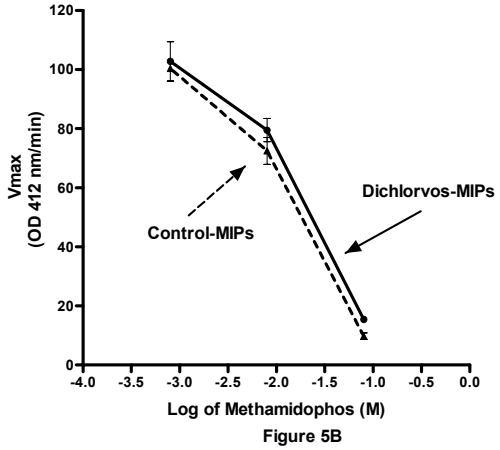


Figure 5B

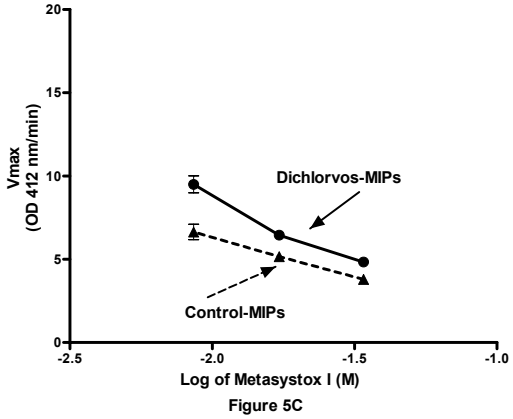


Figure 5C

Figure 5. Binding of Phosdrin (5A), Methamidophos (5B), and Metasystox I (5C) to Dichlorvos-MIPs and control-MIPs. AChE activity was used to measure the binding of these pesticides to the Dichlorvos- and control-MIPs.

Figure 5 shows the specificity of Dichlorvos-MIPs for Dichlorvos over the three other pesticides evaluated in this study (Figure 2). Figure 5A shows that Dichlorvos-MIPs did not bind Phosdrin to any greater degree than it's binding to control-MIPs. Similarly, figures 5B and 5C show that the binding of Methamidophos or Metasystox I to Dichlorvos-MIPs or control-MIPs is identical, and neither of these MIPs bound these pesticides. Thus, the binding of Dichlorvos to Dichlorvos-MIPs is specific.

CONCLUSION

Dichlorvos-MIPs were synthesized that bound to its corresponding template in comparison to control-MIPs. Dichlorvos bound to Dichlorvos-MIPs only when ethanol was used in the binding assays. Dichlorvos-MIPs did not either bind or their binding was minimal to pesticides with similar chemical structures as that of Dichlorvos. Since we prepared MIPs that specifically bound the corresponding template, it might be possible to prepare decontamination MIPs that would not only bind chemical warfare agents, but also detoxify them. Since MIPs are inexpensive and easily synthesized, these polymers could be produced in bulk for a variety of products wherever solid materials could be incorporated.

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